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Duration of viremia following vaccination and smallpox.

by G.P. Alivisatos and M. Violaki-Paraskeva.

Zeit. f. Immunitaetsforschung u. exp. Therapie 117: 230-243 (1959). Partial translation.

It has been known for some time that the disappearance of pathogens from the body does not coincide with cessation of disease. It is generally accepted, however, that viruses circulate in the patient's blood only during the first few days of illness, seldom for longer than one week. Reports of more prolonged viremia were always received with great reservation, although Calmette and Guerin (1) had shown since 1901 that vaccinia virus circulates in the blood for several days without symptoms, and that typical skin eruptions appear only when the animal's fur is shaved or extracted. In addition, Gins (3) demonstrated in 1920 by means of corneal lesions and elicitation of specific keratitis that vaccinia virus circulates silently seven days after intravenous instillatio. Herzberg-Kremmer and Herzberg (7) had similar results in 1930-1931, when they found vaccinia virus in the blood circulation three to ten days after vaccination. On the other hand, Scott and Simon obtained variola virus in 1923 from rhinopharyngeal sputa of two smallpox patients, 23 days after commencement of illness from one, and 31 days after onset from the other, i.e., at a time when all scabs had been shed.

Puntigam and Orth (11) reported recently (1953) that they produced orchitis after intratesticular injection of rabbits with virus-containing blood plasma obtained from calves used in vaccine production; the plasma had been obtained at different intervals after inoculation. Following centrifugation of testicular suspension, these authors found elementary bodies of vaccinia under the electron microscope up to 528 hours (22 days) after injection.

In contrast, the studies (1950) of McCallum, McPherson and Johnstone (10) produced variola virus from the serum or blood plasma of only two out of seven smallpox patients on the third and fourth day after onset, when material was injected into chick embryos. These authors isolated the virus by egg inoculation of rhinopharyngeal secretions from the same eight patients in only two cases, despite the fact that secretions were obtained within 2-6 days after onset. The same authors found it impossibl to isolate variola virus in egg culture, although the secretions were procured from four other patients three to four days prior to appearance of eruption (contact cases), and it is generally accepted that the infectivity of these secretions is highest in the pre-eruptive stage. On the other hand, the pustular pus of all twelve patients contained the virus, as demonstrated by inoculation of chick embryos.

In 1950, Downie, McCarthy and Macdonald isolated the virus by inoculating eggs with serum of variola patients in their first to fourth day of illness; results were positive in four out of seven cases. The rhinopharyngeal secretion of a smallpox patient in the first and second day of illness failed to reveal virus. The authors concludes from these findings that the virus circulates in the blood only during the pre-eruptive stage.

The preceding discussion reveals the divergence of opinion that besets this problem, i.e., the length of time spent by variola or vaccinia virus circulating in the blood after onset of disease. This question is not only of theoretical interest, but is significant from the epidemiological viewpoint and from the aspect of host-parasite relations.

Our studies of this problem proceeded from an observation made by us in 1953 during the investigation (still in progress) of interference phenomena produced by vaccinia virus. We established that the above-mentioned virus may still be cultured from the blood 37 days after intravenous or intracutaneous instillation in rabbits. We must add here that we never used serum or plasma in egg cultures, but always unclotted whole blood (see below), since one of us had found that the majority of organisms are trapped in the mesh of the clot and are transferred to the serum in very small numbers. The same entrapment took place in meshes of milk curd when the survival of Brucella in cheese was studied; the cheese had been made of milk artificially infected with a known quantity of germs.

Viremia after survival of smallpox

Judging by positive rabbit tests and initial positive results of the present study of vaccinated children, we assumed that the conditions were analogous in the case of smallpox, despite the controversies and the impossibility of culturing the virus from the blood save during the first few days of illness. A coincidence confirmed this assumption.

During the night of 6 July 1957, the 29-year-old sailor J. Sk. arrived in Athens by aircraft from Port Sudan. The airport sanitary authorities noted a few sparse, reddish scars in his face and found two scabs on the lower left leg. Status of vaccination: unknown. He was immediately placed in an isolation hospital. The anamnesis revealed that this man had landed at Port Sudan on 8 May 1957 on board a ship sailing from Zedda. On 14 May 1957 he came down with chills, high fever, vomiting, headache and pain in the joints and loin. Two days later he was hospitalized in Port Sudan. On the third day of illness a sparse eruption appeared on his head, descending slowly across his body and becoming pustular. Drying was followed by formation of scabs, which began to drop off until only a few were left on his lower legs by 3 June, the day of discharge from the hospital. Although this man had contracted classical varioloid during the course of a smallpox epidemic in the Sudan, his record listed a noncommittal diagnosis. On 7 July 1957 the State Hygiene Laboratory carried out a blood culture in 12-day chick embryos

by the customary method (blood serum); the result was negative. No scabs were left at this time.

On 21 June 1957, i.e., 38 days after onset of disease and 14-15 days after loss of scabs, when the sailor was scheduled to return home following thorough disinfection, pending examination by the sanitary authorities, we were able to draw blood for water-glycerol fluid, of which lots of 0.1 were injected into two 12-day chick embryos. The result was positive two days later; each membrane contained an average of 40 pustules (Fig. 3).

Now blood serum from the same sample was inactivated for ½ hour at 56°C in order to destroy possible virus particles, mixed thoroughly 1:1 with 1 ml of a 10^{-4} dilution of vaccine (earlier neutralization tests had shown this ratio to be optimal), stored for 2 hours at room temperature, then injected into 12-day chick embryos in lots of 0.1. Forty-eight hours later 0.05 ml of the 10^{-4} dilution of vaccine treated with patient serum had produced an average of 10 pustules, while the control (two chick embryos infected with 0.05 of vaccine diluted to 10-4) had yielded an average of 60 pustules.

The difference between membranes (slightly or severely hemorrhagic, etc.) described previously were again apparent between those subjected to neutralization tests and controls (cf. Fig. 4, 5). This proves that variola virus still circulates in the patient's blood 38 days after onset of disease and 14 days after shedding of crusts, despite the fact that his serum had a relatively high virus-inhibiting potency which permitted the growth of only one-sixth of the virus compared to the control test. The pathogenic power of the virus also seemed to change in the presence of serum, when compared to the control virus.

Discussion

The ~~present~~ experiments repeatedly demonstrated the survival of vaccinia virus in the blood long after the disease process had stopped, including one case of smallpox. If one assumes that an infectious disease is an ecological process aimed not at the destruction of the two competing systems, but possibly at their survival and balanced co-existence, this result is not surprising. The survival of an infectious disease seems to indicate in most cases that the host has become unsuitable for further habitation by the parasite, i.e., that the parasite, while no longer able to propagate in the host's body, does not perish all at once.

Isolated pathogens may exist for extended periods in the host's organism in a state of necrobiosis, i.e., they are able to reproduce, under special conditions, outside the host organism. This type of reservoir is more prevalent than previously assumed. It does not seem to be of great epidemiological importance, but does reinforce the immunity of the host, since daily disintegration of necrobiotic germs acts as a positive impulse on reinforcement of host immunity. In this perspective, the small number of germs present in 0.08 ml of blood must be evaluated

correctly, as must be the observation that the number increased little or not at all during the second transmission. This probably points to a drop in infectivity of vaccine or variola virus; the hypothesis requires confirmation by additional systematic test series.

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